



PATENT  
Atty. Docket No. LEX-003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Gillies *et al.* CONFIRMATION NO.: 9492  
SERIAL NUMBER: 09/256,156 GROUP ART UNIT: 1647  
FILING DATE: February 24, 1999 EXAMINER: Kapust, R.B.  
TITLE: Enhancing the Circulating Half Life of Antibody-Based Fusion Proteins

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION BY STEPHEN GILLIES AND KIN-MING LO UNDER 37 C.F.R. 1.131**

Sir:

We, Stephen Gillies and Kin-Ming Lo, hereby declare as follows:

1. We have reviewed claims 1, 3, 6-8, 10, 29 and 30 as submitted herewith. We are the co-inventors of the subject matter of claims 1, 3, 6-8, 10, 29 and 30 of the above-identified patent application.
2. We make this declaration to establish invention of the subject matter of claims 1, 3, 6-8, 10, 29 and 30 prior to the effective date of International Publication No. WO 97/43316, entitled "Physiologically Active Molecules with Extended Half-Lives and Methods of Using Same," which we understand was published on November 20, 1997.
3. Prior to November 20, 1997, we conceived of a gene construct encoding an IL-2 fusion protein, which included an IgG1 hinge region, an IgG1 CH2 domain with a mutation or a deletion that reduced the binding affinity for an Fc receptor, an IgG1 CH3 domain, and an IL-2 moiety. This construct included a region as claimed in claims 1, 3, 6-8, 10, 29 and 30.

4. We attach as the first page of Exhibit 1 a true copy of laboratory notebook page 2 of Kin-Ming Lo, signed by Kin-Ming Lo, except that dates on the page prior to November 20, 1997, have been removed. We attached as a second page of Exhibit 1 a true copy of laboratory log book page 143, except that the dates on the page, which are all prior to November 20, 1997, have been removed. Exhibit 1 demonstrates that we conceived of the invention of claims 1, 3, 6-8, 10, 29, and 30 prior to November 20, 1997.

5. The laboratory notebook page 2 of Kin-Ming Lo shows a schematic representation of a process for introducing mutations and a deletion into the IgG1 CH2 nucleic acid sequence. As indicated on the top of the page, the purpose of this process is to “mutate the FcR $\gamma$  binding site of  $\gamma_1$  to decrease binding” to the Fc receptor. The first step of the process is shown in the upper portion of the page and is labeled “1<sup>st</sup> set PCR” on the left side of the page. The PCR primers were designed to replace 12 nucleotides of the C $\gamma_1$  CH2 sequence with 9 nucleotides of the C $\gamma_2$  CH2 sequence, thereby modifying the encoded protein to have a reduced affinity for the FcR $\gamma$  receptor. The primers are represented as solid lines on the page, and are identified as 415S, 437A, 1018A, and 1017S. The nucleic acid sequences of primers 1017S and 1018A are provided on laboratory log book page 143. The 1018A and 1017A primers changed the encoded amino acid sequence from Pro<sub>232</sub>-Glu<sub>233</sub>-Leu<sub>234</sub>-Leu<sub>235</sub>-Gly<sub>236</sub>-Gly<sub>237</sub> to Pro<sub>232</sub>-Pro<sub>233</sub>-Val<sub>234</sub>-Ala<sub>235</sub>-Gly<sub>236</sub>.

6. The products of the first PCR step were used in a second step, shown in the center portion of laboratory notebook page 2 of Kin-Ming Lo and labeled “2<sup>nd</sup> set PCR” on the left side of the page. The PCR product was to be cloned into a TA plasmid vector.

7. The bottom portion of laboratory notebook page 2 of Kin-Ming Lo shows a process for constructing a vector containing an IgG1 hinge region, the mutated IgG1 CH2 domain with reduced binding affinity for an Fc receptor, an IgG1 CH3 domain, and an IL-2 moiety. The cloning strategy included ligating a 224 base pair (bp) fragment and a 1100 bp fragment, producing a 1324 bp fragment as shown on the bottom-right portion of the page (the

“1324 bp fragment”). The 224 bp fragment was a Pst I-BspH1 restriction fragment encoding an IgG1 hinge region and a mutated N-terminal portion of C $\gamma$ <sub>1</sub> CH2. This fragment was to be isolated from the TA vector described above. The 1100 bp fragment was a BspH1-Xho I restriction fragment encoding the remaining portion of C $\gamma$ <sub>1</sub> CH2, all of CH3, and the fused IL-2 gene sequence. Thus, the 1324 bp fragment included a region as claimed in claims 1, 3, 6-8, 10, 29, and 30.

8. On or before December 8, 1997, Jie Chen, a technician working under our direction, prepared in the United States a construct including the 1324 bp fragment. We attach as Exhibit 2 true copies of laboratory notebook pages 3, 7, 8, 10, 15, 16, 17 and 18 of Jie Chen, except that the date on laboratory notebook page 3 prior to November 20, 1997, has been removed. Jie Chen signed her laboratory notebook pages 3, 7, 8, 15, 16, 17, and 18. This exhibit is to establish diligence from prior to November 20, 1997, to reduction to practice in the United States of the invention of claims 1, 3, 6-8, 10, 29, and 30.

9. Laboratory notebook page 3 of Jie Chen, dated prior to November 20, 1997, shows a restriction enzyme digest identifying recombinant TA plasmid vectors that include the correctly assembled PCR product described in paragraph 6 of this declaration. The PCR product included an IgG1 hinge region and a mutated N-terminal portion of C $\gamma$ <sub>1</sub> CH2.

10. Laboratory notebook page 7 of Jie Chen, dated November 19, 1997, shows that a DNA sequencing reaction was performed to confirm the sequences of the PCR products in the TA vector in isolates (“clones”) labeled “2” and “3.”

11. Laboratory notebook page 8 of Jie Chen, dated November 20, 1997, shows that DNA sequencing reactions were performed to confirm the sequences of the PCR products in the TA vector in clones labeled “1,” “4,” “5,” and “7.”


12. Laboratory notebook page 10 of Jie Chen indicates that the DNA sequence of clone 5 was determined to be correct. This page also shows a process for preparing the 1324 bp fragment described above using the PCR product from clone 5. The top of the page is dated Friday, November 21, 1997. Because the bottom of the page is undated, we do not know when this page was completed, although it would have been completed no earlier than November 21, 1997.

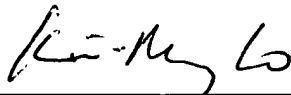
13. Laboratory notebook page 15 of Jie Chen, dated Monday, December 2, 1997, shows another process for preparing the 1324 bp fragment, using the *Drd I* restriction enzyme instead of *BspH1*. The *BspH1* restriction enzyme is sensitive to the methylation status of the DNA, limiting the usefulness of the enzyme. The *Drd I* enzyme is not believed to share this limitation. Thus, between Friday, November 21, 1997, and Monday, December 2, 1997, inclusive, a period that included the Thanksgiving holiday, work was done toward preparation of the 1324 bp fragment; a second, more efficient process for preparing the 1324 bp fragment was identified and planned; and restriction fragments were prepared and analyzed in accordance with the improved process. The photographs on the page show the gel-separated restriction fragments used for preparing the 1324 bp-ligation product.

14. Laboratory notebook pages 16, 17, and 18, of Jie Chen, dated December 3, 4, and 8, 1997, respectively, detail DNA preparations, ligation reactions, transformations, and restriction digests used to prepare and analyze the 1324 bp ligation fragment. On these pages, the fragment encoding the mutant  $C\gamma_1$  CH2 domain was erroneously referred to as " $\gamma_4$ ." Page 18 indicates that a plasmid containing the 1324 bp fragment was successfully prepared on or before Monday, December 8, 1997.

15. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like, so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such

willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 12, 2004   
Stephen Gillies

Date: Jan 12, 2004   
Kin-Ming Lo

Project No. \_\_\_\_\_

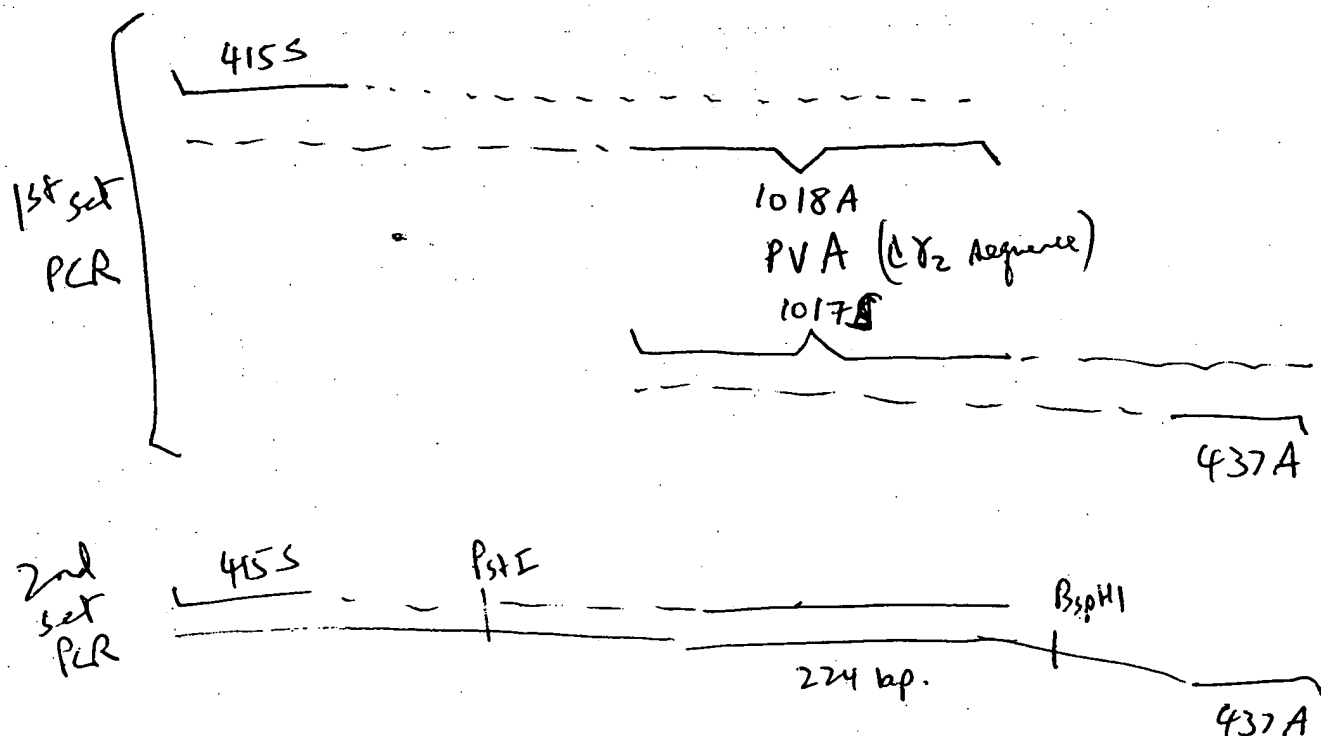
B ok No. \_\_\_\_\_

TITLE

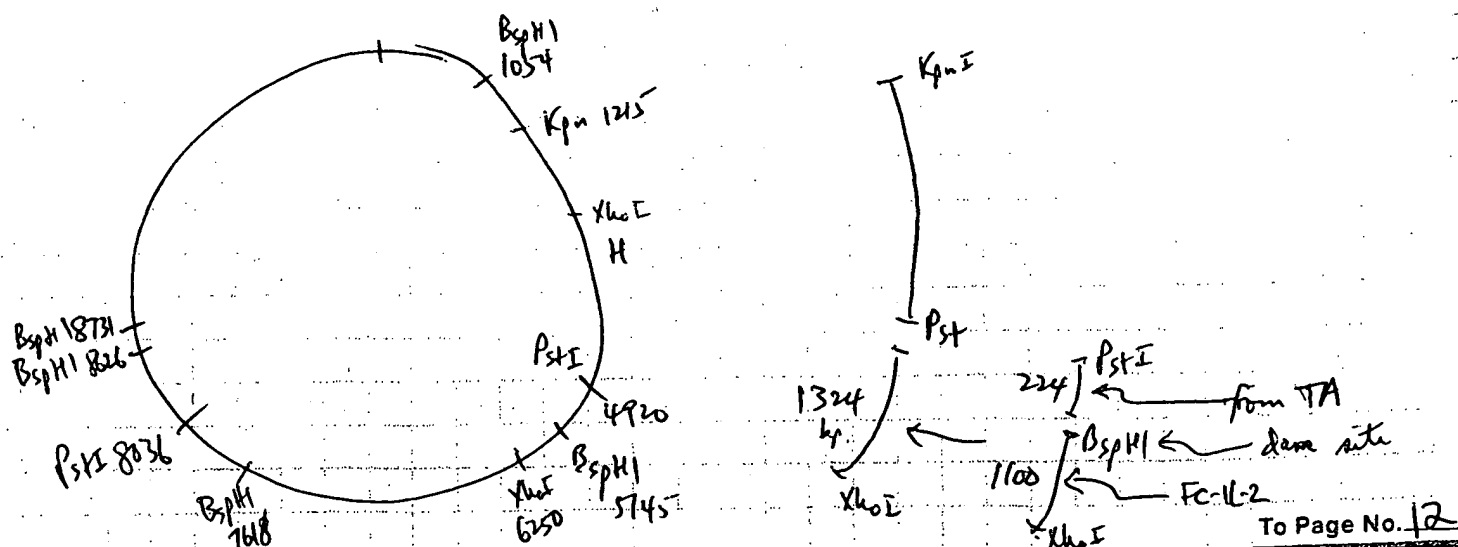
pMHL7 - duKS mutated  $\gamma_1$  - II-2

m Page No. \_\_\_\_\_

We want to mutate the FcR $\gamma$  binding site of  $\gamma_1$  to decrease binding.



We got the PCR product and clone the fragments into TA vector.



To Page No. 12

Witnessed &amp; Understood by me,

Date

12/31/97

Invented by

Record d by 11-M-6

Date

Page No. \_\_\_\_\_

C<sub>8</sub>, delete FcR  
binding**1017S (022129-1)**

36 mer

5'-GCA CCT CCC GTC GCA GGA CCG TCA GTC  
TTC CTC TTCPCR mutagenic  
primer @ 1070

1 OD = 34.6  $\mu$ g = 3196 pmol  
MW = 10824.11 E260 = 312.9 L/mmol-cm  
Tm = 78.5 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 22.6 OD; Desalted

**AMITOF** (617)782-9242 • 1-(800)998-4863 • (617) 782-9352(FAX)**1018A (022129-2)**

35 mer

5'-CGG TCC TGC GAC GGG AGG TGC TGA GGA  
AGA GAT GGC<sub>8</sub>, delete FcR  
bindingPCR mutagenic  
primer @ 1070

1 OD = 31.6  $\mu$ g = 2888 pmol  
MW = 10948.25 E260 = 346.3 L/mmol-cm  
Tm = 79.7 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 21.9 OD; Desalted

**AMITOF** (617)782-9242 • 1-(800)998-4863 • (617) 782-9352(FAX)

1019S

To Page No. \_\_\_\_\_

I have read and understood by me,

Date

Invented by

Date

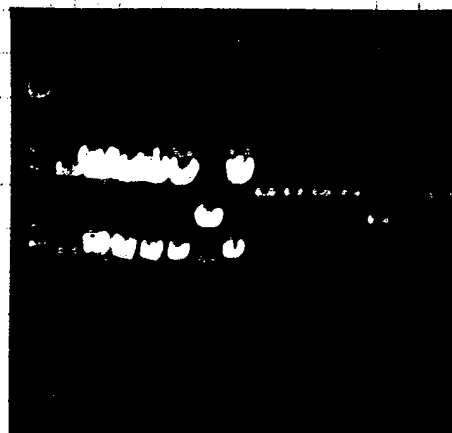
From Page No. \_\_\_\_\_

- mini pre

(pdHL7-huK5-IL2) / 4158-437A in TA #1-14

cut with EcoR-I

#1, 2, 3, 4, 5, 7 are correct.



= Ligation. (in TA)

cDNA: Lox

PCR product : P<sub>2</sub> fragment (Primer F715-R1486)

= Transformation

Lox (P<sub>2</sub> fragment) in TA

To Page No. \_\_\_\_\_

Witnessed & Understood by m ,

Date

Inv nted by

Date

*Shirley Kong*

Record d by

*J. Lecher*



From Page N

11/19/97

- Sequencing Reaction

DNA: huks in TA (11/14/97)

# 2, 3

primer: 1019 S, 1020 A

= mufc - Elisa (Report 11/17/97)

	Optical Density											
	mufc - gmv in CHO						gp120 in CHO					
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.291	0.210	0.157	0.364	0.444	0.503	0.184	0.180	0.212	0.825	0.216	0.276
B	1.219	1.316	0.214	0.229	0.480	0.498	0.170	0.178	0.665	0.372	0.219	0.276
C	1.076	1.194	0.209	0.190	0.482	0.469	0.168	0.170	0.913	0.460	0.219	0.267
D	1.119	1.146	0.265	0.279	0.489	0.466	0.155	0.169	0.497	0.323	0.186	0.222
E	0.946	0.929	0.265	0.240	0.281	0.459	0.161	0.331	0.352	0.349	0.210	0.239
F	0.952	0.838	0.195	0.216	0.461	0.900	0.179	0.759	0.773	0.533	0.193	0.220
G	0.729	0.626	0.161	0.195	0.441	0.850	0.173	0.403	0.482	0.897	0.191	0.223
H	0.560	0.472	0.187	0.483	0.480	0.95	0.175	0.177	0.376	0.754	0.235	0.407

To Page No.

Witnessed &amp; Understood by me,

Date

Invented by

Date

Shirley Kong

11-28-97

Recorded by

Tia Chen

11/19/97

Project No. \_\_\_\_\_  
 Book No. 930131 TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

11/20/97

I. Sequencing Reaction

hunks in TA #1, 4, 5, 7

primer = 1019S, 1020A

II. Read sequencing Gel

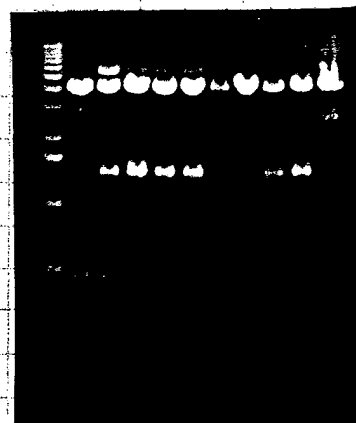
clone #2, 3 of hunks in TA are wrong.

III. miniprep

TC3 (NLS<sup>2-</sup>) in TA #1-10

cut with EcoR.I

#2, 3, 4, 5, 8, 9 are correct.



To Page N. \_\_\_\_\_

Witnessed & Understood by me,

*Shirley Korp*

Date

11-28-97

Inv nt d by

R c rd d by

*Tla chen*

Date

11/20/97

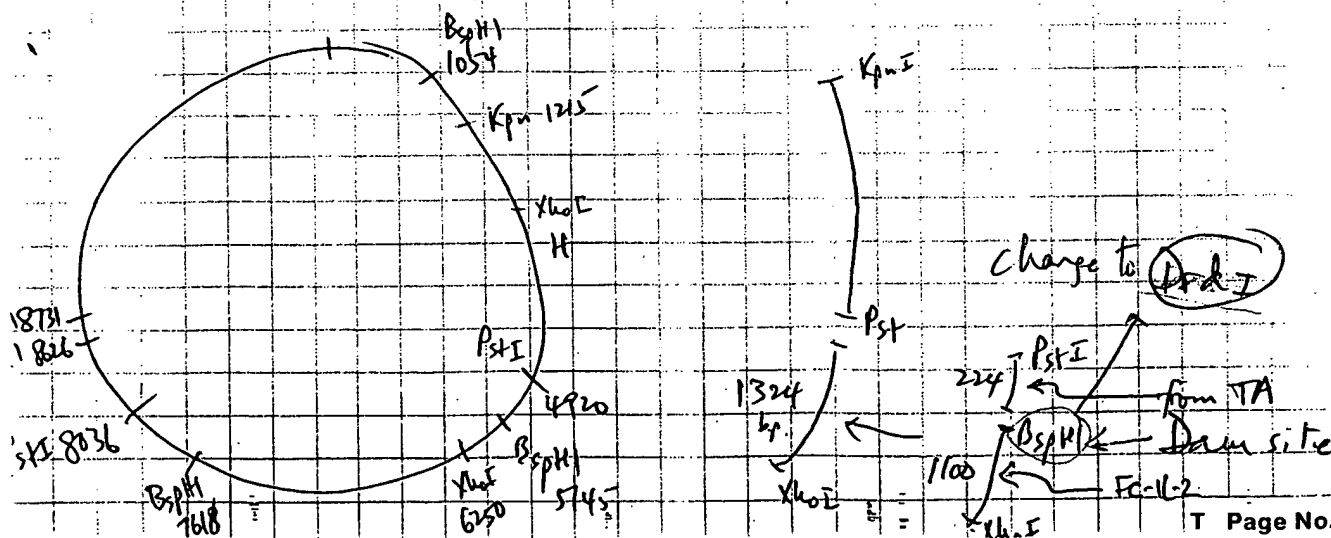
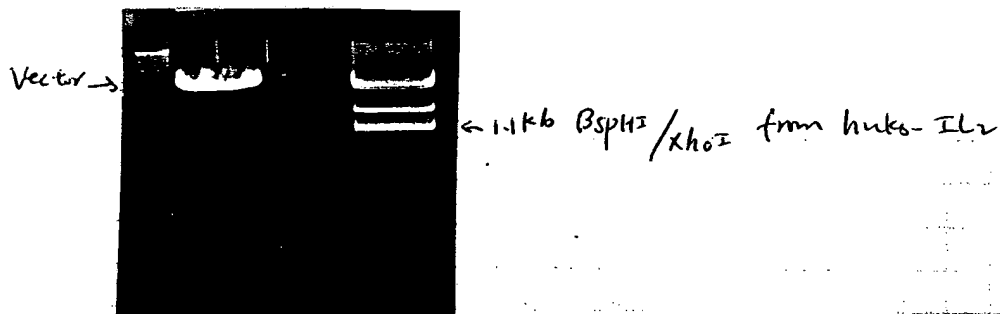
From Page No. \_\_\_\_\_

11/21/97.

- Read sequencing film.

clone #5 of hks in TA is completely correct -

= prepare fragment.

1. vector :  $PstI/XhoI$  in SK2. fragment :  $PstI/BspHI$  from hks in TA  
#5 $BspHI/XhoI$  from hks-IL2

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

12/2/97

- prepare fragment

1. fragment :  $DrdI/PstI$  from hakes in TA #5 (~285bp)← 285bp ( $DrdI/PstI$ )2. fragment :  $DrdI/XhoI$  from pHLg-hu14.18-IL2 (~1kb)←  $DrdI/XhoI$ 

= ligation

cut with  $DrdI$ vector  $PstI/XhoI$  in B5Xinsert  $PstI/DrdI$  (285bp) $DrdI/XhoI$  (1kb)

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

Inv nted by

Date

Shirley Kong

12-28-97

Rec rded by

Tie chen

12/2/97

From Page No. \_\_\_\_\_

12/3/97

## I. sequencing Reaction

Y4 in Ks (  $SphI \rightarrow \text{hindIII linker} \rightarrow XhoI$  )

#4, 6

primer = bob u

## II. miniprep

Y4 in Bst (  $PstI - \text{PrdI} - XhoI$  ) #1-12cut with  $PstI/XhoI$ 

there is no correct clone.

## III. Isolate Genomic DNA

1. MCB Ks-IL<sub>2</sub> N4-627cultured  
(with MTX)2. MCB Ks-IL<sub>2</sub> N58-12-17cultured  
(with MTX)

T Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

Invented by

Dat

Shirley Kong

12-28-97

Recorded by

Jia Chen

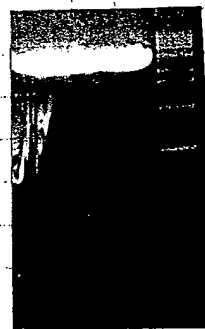
12/3/97

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

12/4/97

- prepare fragment

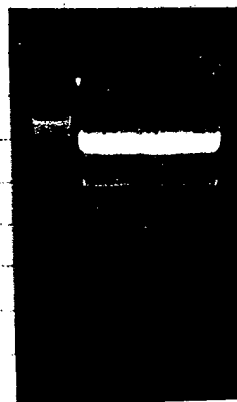
vector  $ks / \text{SmaI} - \text{XhoI}$ 

= Ligation

Repeat

1. Construction of  $CP4FexA$  ( $\text{SphI} \rightarrow \text{hindIII} - \text{XhoI}$ )  
in  $ks$  linker2. Construction of  $FexA-X$  ( $\text{PstI} \rightarrow \text{AflI linker} - \text{XhoI}$ )  
in  $ks$ 

= Transformation

1. Construction of  $CP4FexA$ 2. Construction of  $FexA-X$ 3.  $FexA$  in  $Bsx$  ( $\text{PstI} - \text{PstI} - \text{XhoI}$ )

10. Isolate genomic DNA

hups-IL2 ( $NK-627$ )

(cultured w/o MTK)

hupE18-IL2 ( $N58-12-17$ )

(cultured w/o MTK + P/s)

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by m ,

Shir Kory

Date

12-28

Invented by

Recorded by

Jie chen

Date

12/4/97

From Page No. \_\_\_\_\_

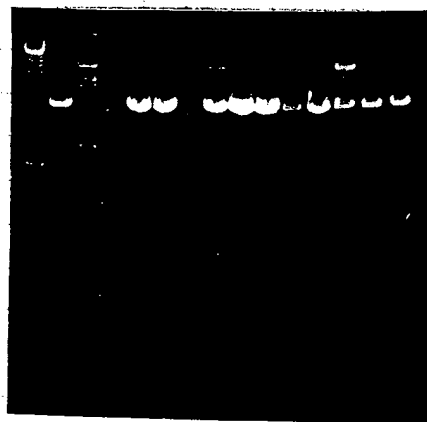
12/8/97

— Mini pre

Y4 in Bsx (PstI - DrdI - XhoI) #1-14

1. Cut with PstI/XhoI

#3, 6 are positive.

2. take #3, 6 to cut  
with DrdI.

This digestion is not  
completed. but we can  
see the insert of 1426bp.  
It means that there is a  
DrdI site between XhoI  
site and PstI.



So #3, 6 are correct.

To Page N \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

12-28

Invented by

Record d by

Tia Chen

Date

12/8/97



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Gillies *et al.* CONFIRMATION NO.: 9492  
SERIAL NUMBER: 09/256,156 GROUP ART UNIT: 1647  
FILING DATE: February 24, 1999 EXAMINER: Kapust, R.B.  
TITLE: Enhancing the Circulating Half Life of Antibody-Based Fusion Proteins

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION OF STEPHEN GILLIES, KIN-MING LO, YAN LAN, AND  
JOHN WESOLOWSKI UNDER 37 C.F.R. 1.131**

Sir:

We, Stephen Gillies, Kin-Ming Lo, Yan Lan, and John Wesolowski, hereby declare as follows:

1. We are co-inventors of the subject matter described and claimed in the above-identified patent application.
2. We have reviewed claim 27 as submitted herewith. We make this declaration to establish invention of the subject matter of claim 27 prior to the effective date of International Publication No. WO 97/43316, entitled "Physiologically Active Molecules with Extended Half-Lives and Methods of Using Same," which allegedly published on November 20, 1997.
3. Prior to November 20, 1997, we conceived of and reduced to practice in the United States an antibody-based fusion protein for administration to a mammal, the fusion protein comprising a variable domain and a portion of an IgG4 CH2 domain, the C-terminus of which was linked by a CH3 domain to the N-terminus of IL-2. The antibody-based fusion



protein had a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to IL-2.

4. We attach as Exhibit A true copies of laboratory notebook pages 137 and 191 of Kin-Ming Lo, signed by Kin-Ming Lo, except that their dates, which are all prior to November 20, 1997, have been removed. These pages are evidence demonstrating that we conceived of and reduced to practice the invention of claim 27 in the United States prior to November 20, 1997.

5. Notebook page 137 shows a schematic representation of an expression vector that was prepared (see below the heading "KS $\gamma_4$ -IL-2"). The vector included an Xho I-Hind III fragment encoding heavy and light chain variable regions derived from the mouse antibody KS-1/4; a Hind III-Nsi I fragment encoding the C $\gamma_4$  region; and a Nsi I-Xho I fragment encoding IL-2. The nucleic acid fragments encoding C $\gamma_4$  and IL-2 were oriented such that, upon expression of the protein, the C-terminus of the C $\gamma_4$  heavy chain is linked to the N-terminus of IL-2.

6. As shown in the table at the bottom of Notebook page 191, experiments were performed using purified KS-1/4-IL-2 fusion proteins containing either C $\gamma_1$  or C $\gamma_4$  heavy chains to compare the *in vivo* circulating half-lives of these fusion proteins. The KS $\gamma_4$ -IL-2 fusion protein was expressed from the vector described on Notebook page 137. The data in the table represent the concentrations of KS $\gamma_1$ -IL-2 and KS $\gamma_4$ -IL-2 measured in blood samples collected from mice at various time points after injection of the respective fusion proteins. KS $\gamma_4$ -IL-2 had a longer circulating half-life *in vivo* than KS $\gamma_1$ -IL-2.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like, so made,

are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 12, 2004 Stephen Gillies  
Stephen Gillies

Date: Jan 12, 2004 Kin-Ming Lo  
Kin-Ming Lo

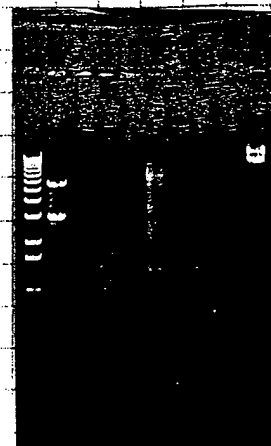
Date: Jan. 12, 2004 Yan Lan  
Yan Lan

Date: Jan 12, 2004 John Wesolowski  
John Wesolowski

From Page No. \_\_\_\_\_

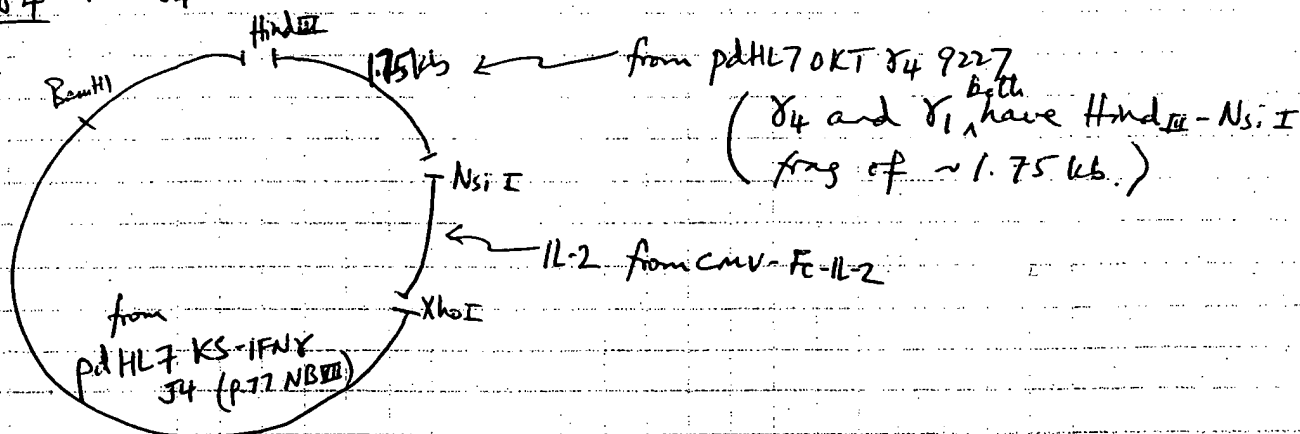
Jie sequenced the ~~OK~~ pDHL2-OKT3 w/ HD6  
Sequence was very clean and it showed the STOP codon followed by  
the 3'-untranslated seq. of the  $\gamma_1$ .  
The size of the OKT3 also looks normal (see p.58 ~~XXXX~~ NB VIII.)

~~82~~  
Summing out OKT3 (the pDHL2 OKT3 <sup>maxi</sup> that was sequenced & used to transfect cells)  
and also pDHL2 OK $\gamma_4$ -9227 w/ the  
following enzymes. The NsiI frag @ 2.8 kb  
increased to ~3.6, showing the presence  
of the 9227 SCA.



Jie did the following:

Y4 KS $\gamma_4$ -IL-2



Jie digested minipreps w/ HindIII + XhoI. #12 dropped out ~2.2 and 8 kb

Jie then digested #12 w/ XbaI 5 + 5.3

EcoRI : 2.7 + ~7.5

BamHI

FspI : 0 0  $\gamma_4$  has FspI ~750 downstream of HindIII

Note that digest of pDHL7 OKT  $\gamma_4$  9227 gave 2 kb + ~8 kb

To Page No. 191

Witnessed & Understood by me,

*T. Chen*

Date

Invented by

Date

Recorded by *K. N. Z.*

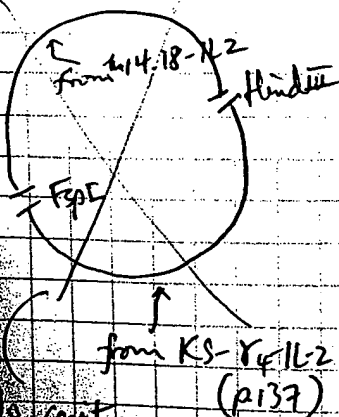
From Page No. 137

You did PK  
studies comparing  
KS-IL2 vs  
KS 84-IL2  
(For construction,  
See p.137)

Conclusion:  
KS 84-IL2 has  
a much longer  
half-life and  
the 84-IL2  
is intact.

You digested the  
KS 84-IL2  
KS 84-IL2  
protein w/ w/  
plasmin.

SM will make  
the 14-18-84-IL2



Witnessed & Understood by me,  
[Signature]

H<sub>2</sub>FL ELISA

H-FL ELISA 3.0 mg/l

Mouse #	30'	1h	2h	4h	8h	24h	48h	72h
1	10.5	13	9		5.4	4.1		
2	12.6	9	9		5.5	4.1		
3	12.0	7.6		6.4		5.2		
4	19.5	12.4		7.7		2.0		
(Mean)	13.6	10.5	9	7.1	5.4	3.9		
5	4.3	2.1	1.2		1.0	0.2		
6	4.4	2.4	1.9		1.0	0.5		
7	4.3	2.0		1.1		0.5		
8	4.9	2.4		1.7		0.5		
(Mean)	4.5	2.2	1.5	1.4	1.0	0.43		

starting material: KS-84-IL2 108 mg/l-e 103 mg/l-e  
KS-11-IL2 124 mg/l-e 100 mg/l-e

H-FL ELISA 1.86 mg/l-e

H<sub>2</sub>FL / H<sub>2</sub>FL ELISA

Mouse #	30'	1h	2h	4h	8h	24h	48h	72h
1	9.2	10.3	8.2		5.5	3.3		
2	11.7	8.4	6.8		4.6	3.3		
3	9.3	6.3		6.0		5.0		
4	22.4	10.2		7.6		1.5		
(Mean)	10.1	8.8	7.5	6.8	5.1	3.3		
5	3.6	1.4	0.65		0.65	0		
6	3.4	1.3	1.0		0.60	0.05		
7	3.0	1.2		0.7		0.09		
8	3.6	1.3		0.9		0.1		
(Mean)	3.4	1.3	0.83	0.8	0.63	0.06		

starting material: KS-84-IL2 124 mg/l-e 103 mg/l-e

To Page No. 192

Date

Invented by

Date

Recorded by

[Signature]